

IONOPHORE-LIKE ACTION OF LIENOMYCIN ON ENERGIZED MEMBRANE OF RAT-LIVER MITOCHONDRIA

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1. Introduction

Lienomycin is a new polyene antibiotic produced by *Actinomyces distatochromogenes* var. *lienomycini*, exhibiting antifungal, antibacterial and antitumor activity [1–4]. It changes the plasma membrane permeability characteristics of sensitive organisms [5].

The present report deals with so far unobserved sensitivity of rat-liver mitochondria to lienomycin. The antibiotic stimulates mitochondrial State 4 respiration and causes release of protons. The stimulatory effect of lienomycin on respiration is dependent on cation composition of the medium and is several-fold higher in the presence of Tris than K^+ . In iso-osmotic Tris–acetate and diethanolamine acetate lienomycin induces swelling of energized mitochondria.

The results obtained indicate that lienomycin exerts ionophore-like action on rat-liver mitochondria in energized state, increasing the permeability of energized mitochondrial membrane to substituted ammonia-cations, such as Tris and diethanolamine.

2. Methods

Rat-liver mitochondria were prepared according to the procedure of Loewenstein et al. [6] with the omission of digitonin step. Oxygen-uptake and pH were monitored as described previously [7]. Swelling of mitochondria was measured at 620 nm in SP 800 Unicam Spectrophotometer. Mitochondrial protein was determined by the biuret method [8] and bovine serum albumin (Sigma) was used as standard. Commercially available sucrose was passed through the H^+ -form

amberlite IRC-50 in order to remove the cations and it was buffered with Tris–Cl, pH 7.3.

Lienomycin was obtained from the Institute for New Antibiotics, Acad. Med. Sci., Moscow, USSR. It was dissolved in dimethylformamide immediately before experiments. Decahydrolienomycin and *N*-glycosyl-derivatives of aureofacin and polyfungin were synthesised in our laboratory.

3. Results and discussion

Lienomycin increased the rate of State 4 respiration of rat liver mitochondria (fig.1). The increased oxygen-uptake was accompanied by proton-ejection from mitochondria when antibiotic was introduced into medium after State 3 → State 4 transition (fig.1A) as well as when lienomycin was added to the medium without ADP (fig.1B).

The presence of five conjugated double-bonds in the molecule of lienomycin was indispensable for biological activity of the compound, as decahydrolienomycin was unable to alter respiratory activity of mitochondria. *N*-Glycosyl derivatives of polyene macrolide antibiotics polyfungin and aureofacin, chemically related to lienomycin, had no effect on mitochondrial respiration (table 1).

Previous observations on the induction by lienomycin of changes of cytoplasmic membrane permeability to monovalent-cations in bacteria, yeasts and erythrocytes [5] as well as the analogies of the effects of lienomycin and members of the valinomycin group in mitochondria suggest that lienomycin-stimulated respiration and the H^+ -production could be consequent

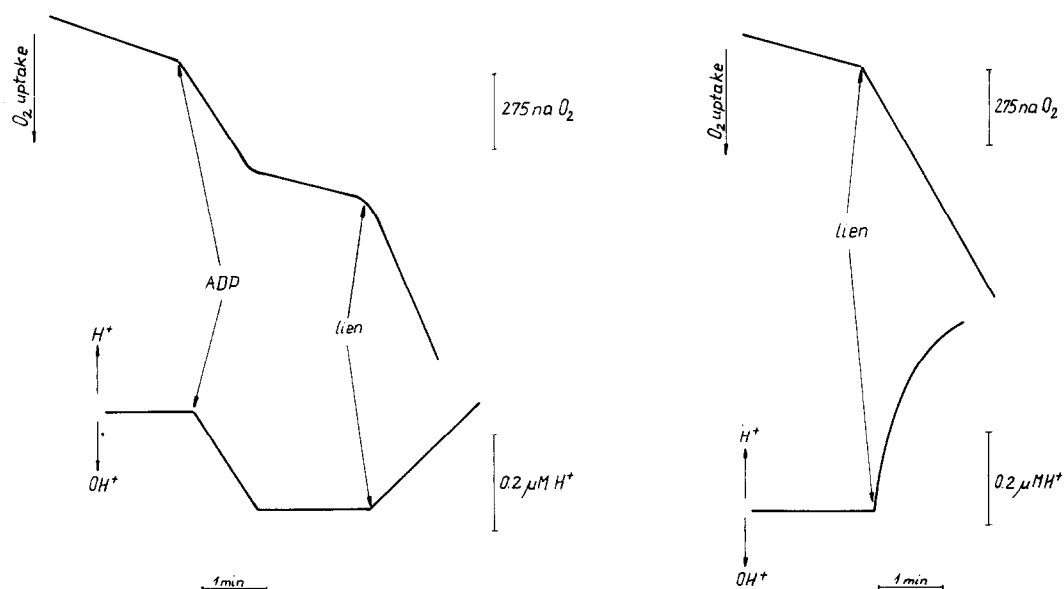


Fig. 1. Effect of lienomycin on respiration and proton-movement in rat-liver mitochondria. Mitochondria (1.8 mg protein) were added into 2.0 ml of medium, pH 7.3, containing: sucrose 200 mM, KCl 10 mM, MgSO_3 3 mM, Tris-chloride 10 mM, Tris-succinate, potassium glutamate and potassium phosphate 5 mM each. Lienomycin (lien) 2 μg , ADP 100 μM . Temperature 25°C.

on increasing permeability of mitochondrial membrane to ions.

The question should be raised as to whether lienomycin acts like an ionophore or as a protonophore (uncoupler). Figure 2 shows that lienomycin-stimulated respiration was not affected by gramicidin (fig. 2A) but was inhibited by dinitrophenol (DNP) (fig. 2B). It indicates ionophore-like action of lienomycin. The

inhibition of lienomycin stimulated respiration by DNP resembles the properties of the valinomycin-type ionophores. The latter compounds, increasing biological membrane permeability to cations but not to protons, accelerate mitochondrial oxygen uptake rate and this stimulation is inhibited by proton-conducting compounds (for review see [9]).

The influence of lienomycin on passive ion-perme-

Table 1
Effect of lienomycin, decahydrolienomycin, *N*-glycosyl polifungin and *N*-glycosyl aureofacin on State 4 respiration of rat-liver mitochondria

Antibiotics	na O_2 /min	
	Tris-succinate	Potassium glutamate
Control	90	60
Lienomycin	380	200
Decahydrolienomycin	90	60
<i>N</i> -glycosyl polifungin	90	60
<i>N</i> -glycosyl aureofacin	90	60

Mitochondria (7.0 mg protein) were added to 3.5 ml of medium containing: KCl 15 mM, EGTA 0.1 mM, Tris-chloride 50 mM, MgSO_4 2.5 mM, potassium phosphate 5 mM. Antibiotic concentration was 10 $\mu\text{g}/\text{ml}$. Final pH was 7.3. Temperature was 25°C.

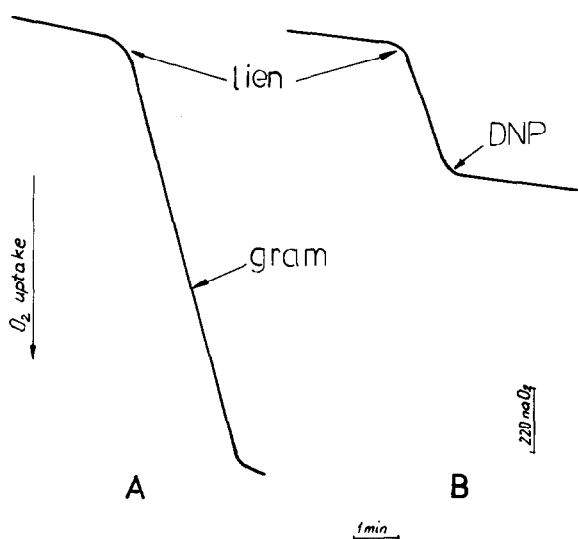


Fig.2. Influence of gramicidin and DNP on lienomycin-stimulated respiration. Mitochondria (7.0 mg protein) were added into 3.5 ml of medium, pH 7.3, containing: sucrose 300 mM, MgSO_4 2.5 mM, EGTA 0.1 mM, potassium glutamate and potassium phosphate 5 mM each. Lienomycin (lien) 3.5 μg , gramicidin (gram) 3.5 μg and dinitrophenol (DNP) 100 μM were added as indicated in the figure. Temperature 23°C.

ability was studied with non-respiring mitochondria by using an osmotic swelling system. Figure 3 shows that unlike valinomycin, lienomycin, at concentration stimulating respiration, does not produce swelling of mitochondria in iso-osmotic potassium acetate. Swelling also does not occur when lienomycin has been added in combination with valinomycin or DNP. The lack of induction by lienomycin of passive K^+ -permeability can be due either to different from valinomycin ion-selectivity or to the requirement of metabolic energy for interaction of the antibiotic with the mitochondrial membrane. This problem will be a subject of further investigations.

More detailed studies on the ability of lienomycin to alter the mitochondrial respiratory activity were performed in the media containing sucrose in addition to substrate Tris, K^+ or Na^+ as the only cation. Results presented in fig.4 indicate, that sensitivity of mitochondrial respiratory metabolism to lienomycin is influenced by the cation composition of the medium. Stimulation by lienomycin of oxygen uptake in Tris-containing medium was several-fold higher than in the presence of potassium. In the presence of sodium no stimulation was observed. In the Tris-containing medium the rate of respiration increased, in approxi-

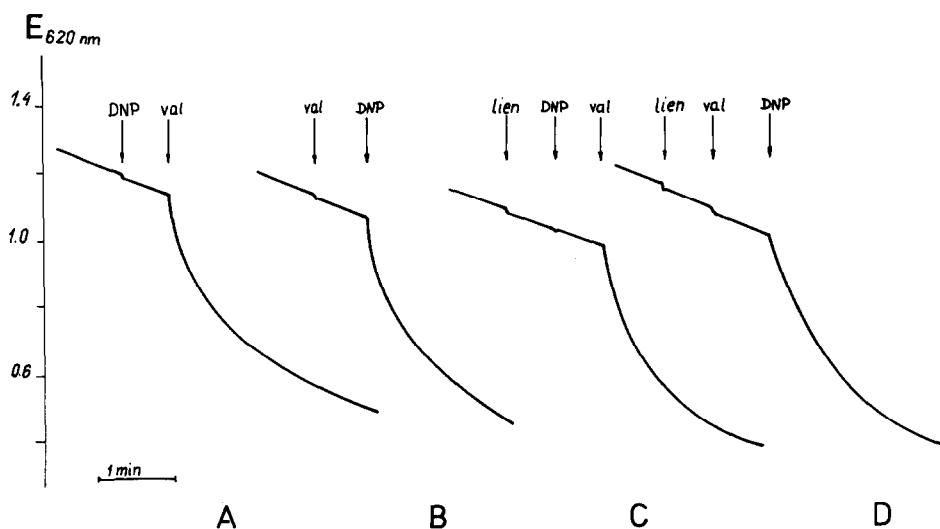


Fig.3. Effect of lienomycin on passive swelling of rat-liver mitochondria in potassium acetate. Mitochondria (2.0 mg protein) were added into 2.5 ml of medium, pH 7.3, consisting of: potassium acetate 100 mM, EGTA 0.1 mM and 5 μg rotenone. Lienomycin (lien) 1.25 μg , valinomycin (val) 2.5 μg and DNP 100 μM were added as the arrows indicate. Temperature 24°C.

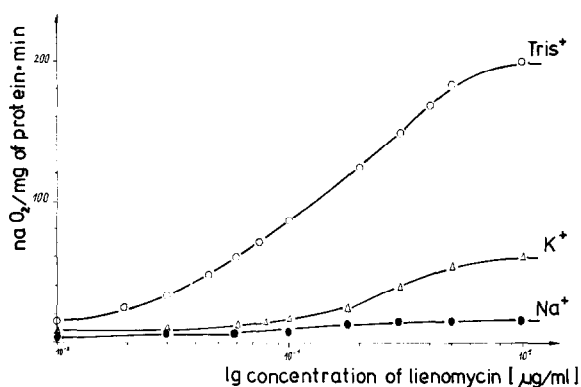


Fig. 4. Relation between lienomycin concentration, cation composition of the medium and mitochondrial respiration. Mitochondria (4.8 mg protein) were added to 3.5 ml of medium, pH 7.3, containing in addition to sucrose 200 mM and EGTA 0.1 mM: Tris-chloride 10 mM, Tris-glutamate, Tris-succinate and Tris-phosphate, 5 mM each. In potassium or sodium containing medium Tris-salts were replaced by potassium- or sodium-salts, respectively. Temperature 23°C.

mately linear fashion, with increasing concentration of the antibiotic and showed the saturation characteristic above 0.5 $\mu\text{g/ml}$.

These findings suggest that lienomycin increases the

permeability of mitochondrial membrane to Tris more effectively than to K^+ . The fact that lienomycin increases the mitochondrial membrane permeability to Tris is also indicated by rapid swelling of energized mitochondria in iso-osmotic Tris-acetate under the influence of this antibiotic. Swelling was supported either by electron-transport or by exogenous ATP. Uncoupling agent, DNP, inhibited the swelling resulting from the action of lienomycin (fig. 5). The same type of behaviour was observed when Tris-acetate was replaced by diethanolamine acetate (fig. 6). However, as with potassium acetate, resting mitochondrial swelling with Tris-acetate did not occur.

The results obtained indicate that lienomycin is a compound of a new type of chemical structure with ionophore-like action on the mitochondrial membrane. In energized conditions, possibly essential membrane-antibiotic interaction, lienomycin at very low concentrations facilitates the entry of substituted ammoniacations into mitochondria. These properties make this antibiotic useful in study of the correlation between energy conserving reactions and permeability-barrier function of the mitochondrial membrane.

It should be pointed out that there is significant difference in biological properties of nonmacrolide

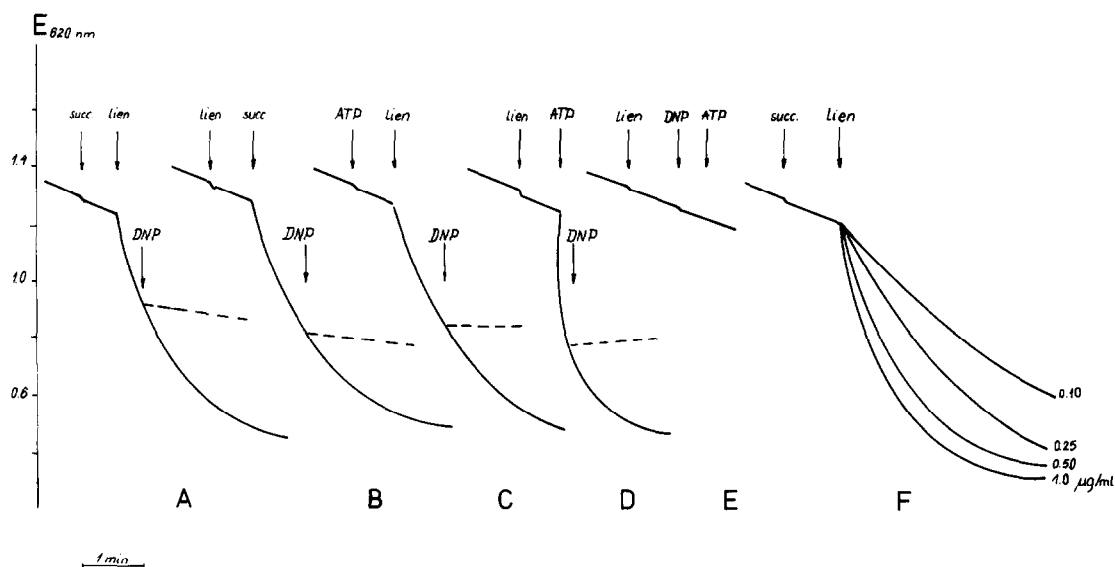


Fig. 5. Induced by lienomycin energy dependent swelling of mitochondria in Tris-acetate. Mitochondria (2.0 mg protein) were added into 2.5 ml of medium, pH 7.3, consisting of: Tris-acetate 100 mM, EGTA 0.1 mM, rotenone 5 μg . Other additions were: Tris-succinate (succ) 5 mM, ATP 5 mM, lienomycin (lien) 1.25 μg and DNP 100 μM . Temperature 24°C. (—) Without DNP, (---) with DNP.

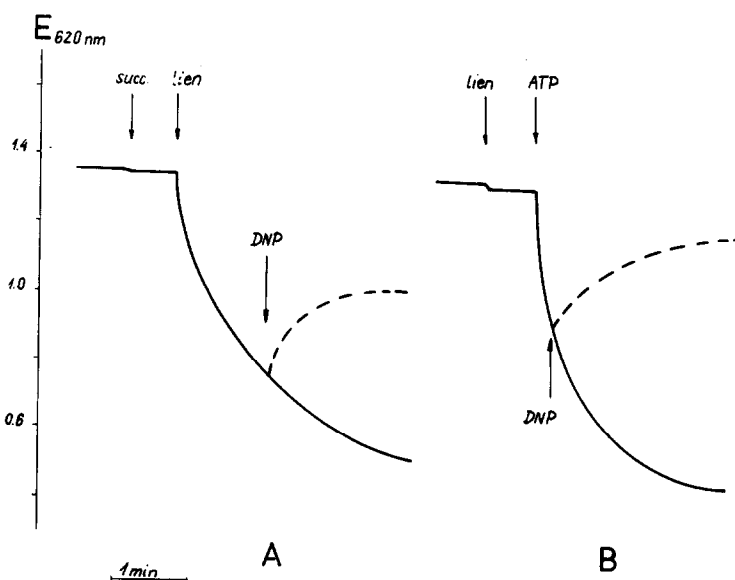


Fig.6. Induced by lienomycin energy-dependent swelling of mitochondria in diethanolamine acetate. Mitochondria (2.0 mg protein) were added to 2.5 ml of medium, pH 7.3, containing: sucrose 100 mM, diethanolamine acetate 70 mM, EGTA 0.1 mM and rotenone 5 μ g. Tris-succinate (succ) 5 mM, ATP 5 mM, lienomycin (lien) 2.5 μ g, DNP 0.1 mM were added as indicated in the figure. Temperature 24°C. (—) Without DNP, (---) with DNP.

polyene-lienomycin and chemically related groups of polyene macrolide antibiotics. The latter compounds, except filipin [10], have no effect on mitochondria and induce permeability changes only in the cytoplasmic membrane of eukariotic organisms due to the formation of complexes with sterol components of membrane. However, lienomycin increases membrane permeability not only in sterol-containing organisms but also in bacteria. It could be expected that the phospholipid constituents of the membranes, but not the sterols, are critical for the interaction of lienomycin with biological membranes. This could explain the sensitivity of mitochondria to this antibiotic.

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